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Stem cell biology and neurodegenerative disease

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The fundamental basis of our work is that organs are generated by multipotent stem cells, whose properties we must understand to control tissue assembly or repair. Central nervous system (CNS) stem cells are now recognized as a well-defined population of precursors that differentiate into cells that are indisputably neurons and glial cells. Work from our group played an important role in defining stem cells of the CNS. Embryonic stem (ES) cells also differentiate to specific neuron and glial types through defined intermediates that are similar to the cellular precursors that normally occur in brain development. There is convincing evidence that the differentiated progeny of ES cells and CNS stem cells show expected functions of neurons and glia. Recent progress has been made on three fundamental developmental processes: (i) cell cycle control; (ii) the control of cell fate; and (iii) early steps in neural differentiation. In addition, our work on CNS stem cells has developed to a stage where there are clinical implications for Parkinson's and other degenerative disorders. These advances establish that stem cell biology contributes to our understanding of brain development and has great clinical promise.

Keywords: stem cells; synapses; Parkinson's disease

It has been many years since evidence was first obtained for multipotent precursor cells in the vertebrate and invertebrate nervous systems (Nieuwkoop 1952; Ready *et al.* 1976; Le Douarin 1980). In our group, *in vivo* studies were first used to identify CNS stem cells (Hockfield & McKay 1985; Frederiksen & McKay 1988; Lendahl *et al.* 1990). Identification of the cell was rapidly followed by evidence for the developmental plasticity of CNS stem cells obtained by transplantation into ectopic sites in the CNS (Frederiksen *et al.* 1988; Renfranz *et al.* 1991; Brustle *et al.* 1995; Vicario-Abejon *et al.* 1995). The potential of CNS stem cells expanded *in vitro* was also defined (Temple 1989; Cattaneo & McKay 1990; Reynolds & Weiss 1992; Davis & Temple 1994). The molecular mechanisms controlling CNS stem cell numbers and fate choice are now the focus of work in many laboratories using both *in vivo* and *in vitro* systems (Isaka *et al.* 1999; Mitsuhashi *et al.* 2001; Sun *et al.* 2001; Hermanson *et al.* 2002; Petersen *et al.* 2002; Turnley *et al.* 2002).

During development, precursor cells in the nervous system assume a positional identity within a spatial coordinate system that has a dorsal–ventral, rostral–caudal and left–right axis (Altmann & Brivanlou 2001). When these cells subsequently undergo mitotic arrest they rapidly acquire characteristics of a specific terminal fate. The differentiation of stem cells *in vitro* shows that many of these specific features occur in the absence of the precise organization that occurs *in vivo*. The self-organization shown during stem cell differentiation raises the intriguing question of how these concerted responses are achieved.

We have used two different techniques to study the molecular basis of stem cell commitment. In the first approach, we have studied the action of known morphogens on stem cells *in vitro* and *in vivo*. We propose that the morphogenic actions of BMPs on stem cell proliferation, identity and terminal differentiation are achieved by a feed-forward switch in receptors expressed by stem cells (figure 1; Panchision *et al.* 2001).

In the second approach, we identified a new gene, NS, that is expressed in stem cells in different tissues and regulates the cell cycle (figure 2; Tsai & McKay 2002). This work indicates that NS is part of a switch in the control of p53 when proliferating stem cells transit into committed progenitor cells. p53 regulates apoptosis and is mutated in most human cancers (Bullock *et al.* 2000).

Evidence from our laboratory that stem cells can make functionally mature progeny has contributed importantly to the interest in these cells. Neurons are perhaps best defined by their ability to form synapses. This is not a simple cell-autonomous process, as interactions with astrocytes are critical to synapse formation (figure 3; Blondel *et al.* 2000; Collin *et al.* 2001; Mauch *et al.* 2001; Mozhayeva *et al.* 2002). In the presence of astrocytes, hippocampal stem cells from the foetal and adult nervous system generate neurons with glutamat- and GABA-ergic synapses (Vicario-Abejon *et al.* 2000; Song *et al.* 2002). Behavioural and electrophysiological tests demonstrate that functional dopaminergic neurons can also be derived from expanded midbrain precursors (Studer *et al.* 1998) and from ES cells (Lee *et al.* 2000; Kim *et al.* 2002). In addition, motor neurons (Wichterle *et al.* 2002) and functional glia (Brustle *et al.* 1999) have been obtained from ES cells. These experiments indicate that stem cells expanded *in vitro* can differentiate to functional progeny (figure 4).

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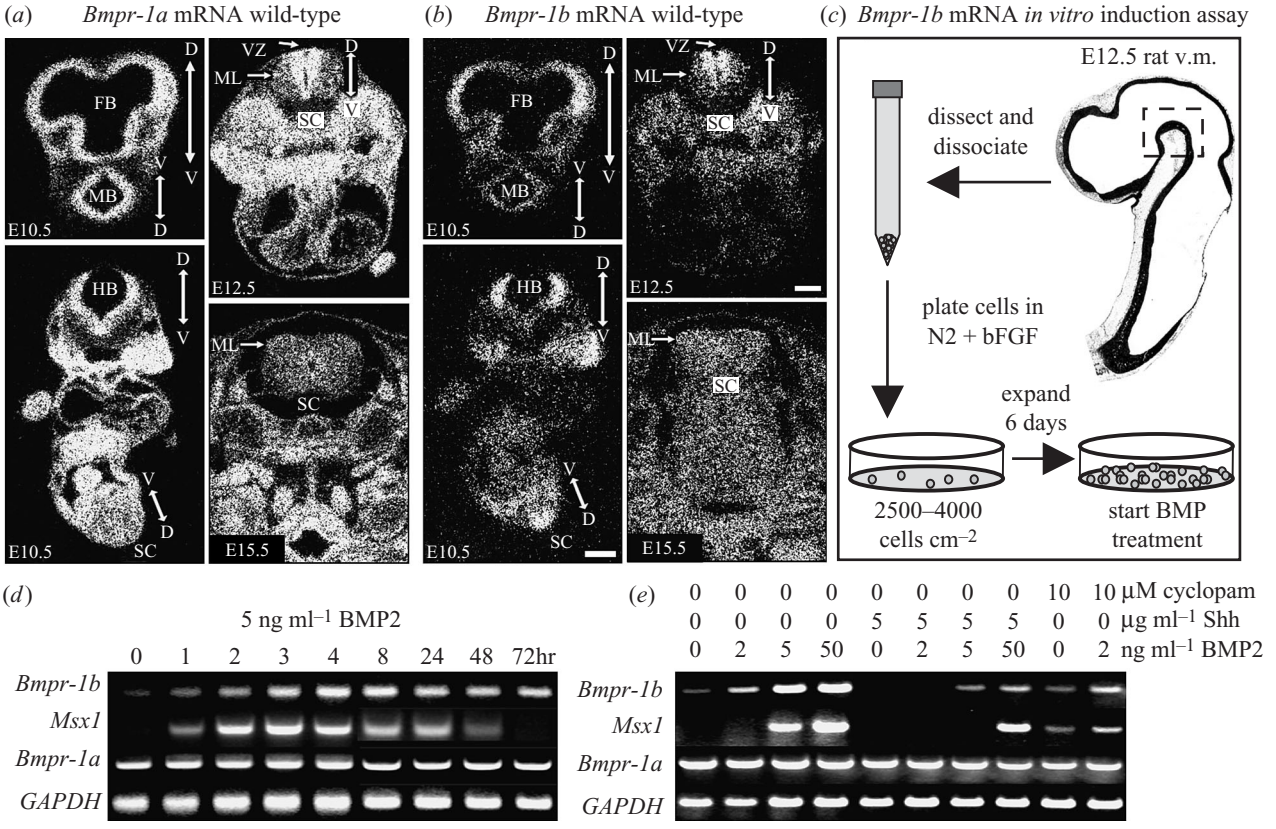

Figure 1. Bmpr-1b is expressed in the dorsal portion of Bmpr-1a expression domain and is induced by BMP2 and BMPR-1A activation. (*a*) *In situ* hybridization for Bmpr-1a on wt embryos showing normal expression pattern during development; note ubiquitous expression in proliferating ventricular zone and lack of expression in differentiated mantle layer. Dorsal–ventral axis of neural tube is indicated by arrows. (*b*) *In situ* hybridization for Bmpr-1b on wt embryos showing normal expression pattern during development; note that expression is restricted to dorsal proliferating neuroepithelium and is not expressed in post-mitotic mantle layer. All images in (*a*,*b*) are from adjacent sections except for E15.5 sections. (*c–e*) Induction of Bmpr-1b expression by BMP2 and caBmpr-1a. (*c*) Diagram showing isolation and culturing of E12.5 rat ventral midbrain stem cells. (*d*) rtPCR of E12.5 ventral midbrain stem cells showing induction of Bmpr-1b and Msx1 within 2–4 h of treatment with 5 ng ml^{-1} BMP2; Bmpr-1a and GAPDH expression are unchanged. (*e*) Induction of Bmpr-1b and Msx1 at 4 h is inhibited by Shh (sonic hedgehog) co-treatment; inhibition of Shh by cyclopamine promotes induction of Bmpr-1b and Msx1. The induction of Bmpr-1b is also seen with expression of activated Bmpr-1a constructs (not shown). Abbreviations: D, dorsal; V, ventral; FB, forebrain; MB, midbrain; HB, hindbrain; SC, spinal cord; VZ, ventricular zone; ML, mantle layer; v.m., ventral midbrain. Bar, 500 μm for E10.5 images in (*a*) and (*b*); bar, 500 μm for E12.5 and E15.5 images in (*a*) and (*b*). Data reproduced from Panchision *et al.* (2001).

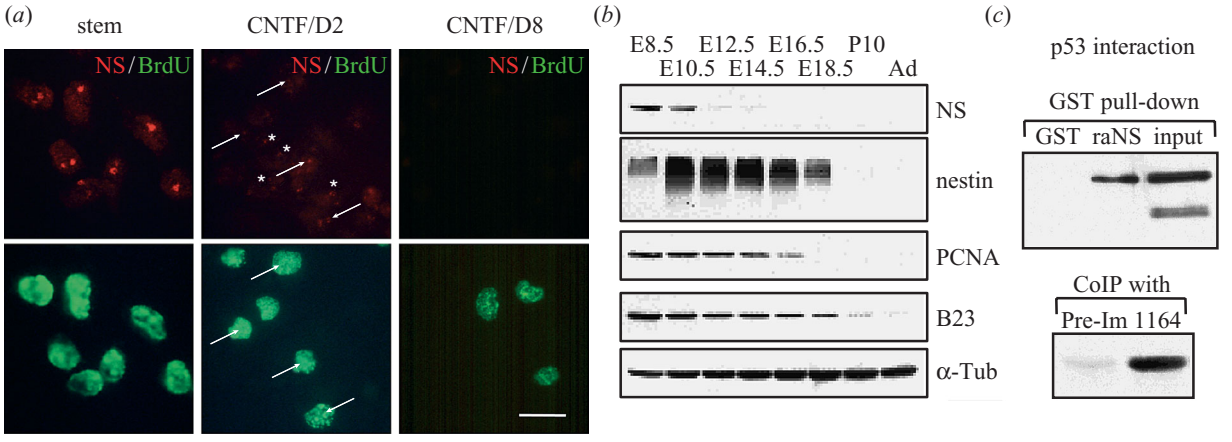

Figure 2. NS identifies an early proliferating CNS precursor. (*a*) NS (red) co-expresses with S-phase cells as measured by BrdU (bromodeoxy uridine) incorporation (green). CNTF (ciliary neurotrophic factor) treatment for 2 days downregulates NS expression even while cells remain cycling; cell cycle arrest is seen by 8 days. (*b*) NS is expressed in early neural tube and is downregulated by E12.5; by contrast, nestin and PCNA expression remains strong until E16.5–18.5. (*c*) NS interacts with p53. Reproduced from Tsai & McKay (2002).

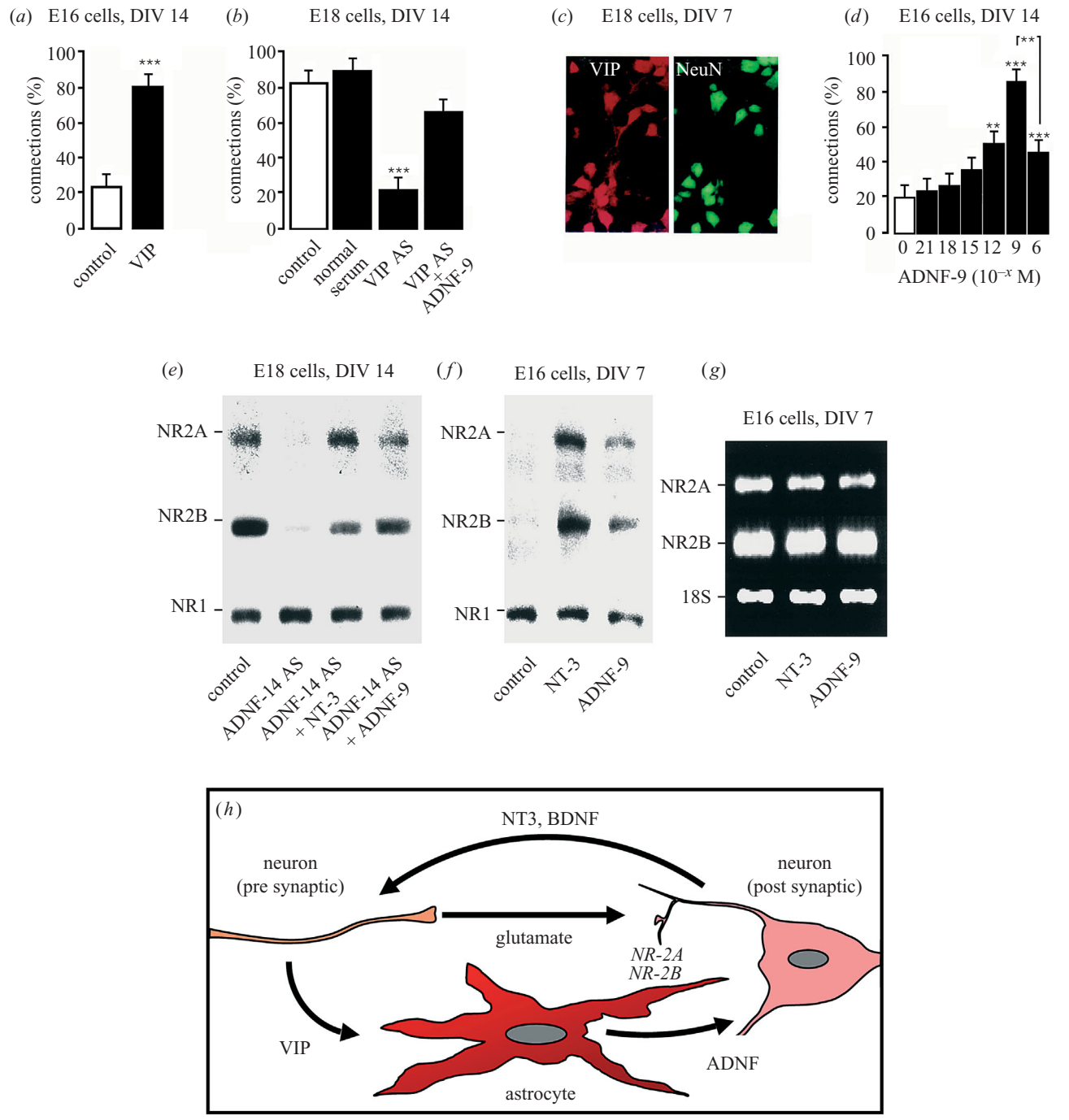

Figure 3. VIP (vasoactive intestinal polypeptide) and ADNF (activity-dependent neurotrophic factor)-9 regulate synaptogenesis in cultures of embryonic hippocampal neurons, whereas ADNF-9 and NT3 regulate NMDA receptor subunit expression. (*a*) Two-week treatment of E16 cultures with 10^{-10} M VIP caused a marked increase in the level of synaptic connectivity. (*b*) Two-week treatment with a VIP antiserum prevents the spontaneous formation of active synapses in E18 cultures, an effect that is reversed by co-treatment with 10^{-9} M ADNF-9. (*c*) Expression of VIP by E18 hippocampal neurons in culture. Cells were grown for 7 days, fixed and immunostained for the expression of VIP and NeuN. Magnification, ×63. The same pattern was obtained after staining three independent cultures. (*d*) Dose–response effect on connectivity in E16 cultures treated for one week with ADNF-9. $^{**}\ p < 0.01$; $^{***}\ p < 0.001$, *t*-test. Results are mean ± s.e.m. of 10–23 cells from at least two independent cultures. DIV, days *in vitro*. (*e–g*) Expression of NMDA receptor subtypes by hippocampal neurons in culture. Proteins from E18 (*e*) and E16 (*f*) hippocampal cultures were analysed by immunoblotting. The amount of each receptor subunit was determined using antibodies specific to each subunit. In E18 neurons after two weeks in culture (*e*), ADNF-14 AS (anti-serum) treatment resulted in a marked decrease in the expression of the NMDA receptor subunits NR2A and NR2B when compared with control cells. This effect of ADNF-14 AS could be prevented by co-treatment with 10^{-8} M ADNF-9 or 20 ng ml^{-1} NT-3. In E16 neurons after one week in culture (*f*), treatment with 10^{-9} M ADNF-9 or 20 ng ml^{-1} NT-3 resulted in a marked increase in NR2A and NR2B expression. (*g*) Expression levels of NT-3 were assayed in E16 cultures after one week in culture using a semiquantitative reverse transcription–PCR method. Treatments for 48 h with 10^{-9} M ADNF-9 or 20 ng ml^{-1} NT-3 failed to induce any significant changes in NR2A or NR2B mRNA levels. Amplification of ribosomal RNA 18S was used as control. (*h*) Model for the development and control of synaptic activity by neurotrophins, VIP and ADNF based on data from Vicario-Abejon *et al.* (2000) and Blondel *et al.* (2000).

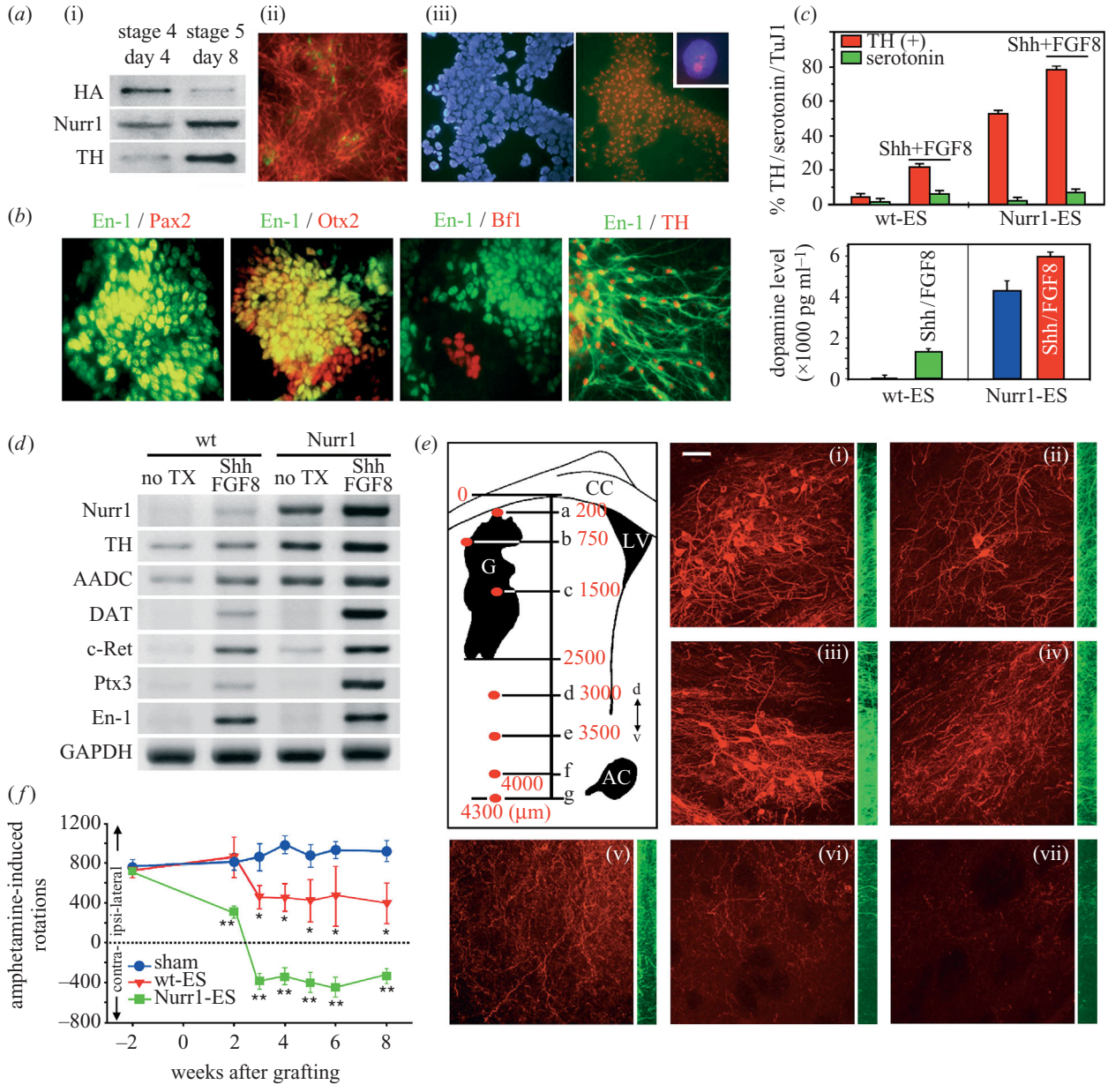

Figure 4. Dopamine neurons derived from ES cells function in an animal model of Parkinson's disease. (*a*) (i) Immunoblot analysis for transfected Nurr1 (HA), total Nurr1 and TH at stages 4 and 5. (ii) Differentiation of Nurr1-tranfected ES cells into TH+ (red) and serotonin+ (green) neurons at stage 5. (iii) Nurr1 overexpression by anti-HA immnuostaining (red) at stage 1 (DAPI, blue; TH, green; inset: co-localization of Nurr1 with DAPI at higher magnification). (*b*) Nurr1-expressing stage four cells show markers of midbrain precursors; En1 co-localizes with Pax2 and Otx2. By contrast, few Bf1+ (forebrain) cells were En1+. After differentiation at stage 5, most TH+ neurons express En-1. (*c*) a, wt- and Nurr1-ES cells were differentiated using five-stage protocol. Generation of TH+ and serotonin+ neurons was measured at stage 5, day 10. Generation of TH+ neurons is enhanced in wt- and Nurr1-ES cells by Shh (sonic hedgehog) and FGF8 (fibroblast growth factor 8) at stage 4. (ii) PLC quantification of dopamine release by stage 5 ES cells after depolarization in Hank's balanced salt solution (56 mM KCl) for 15 min. (*d*) Expression analysis of genes involved in midbrain neuron development and function by RT-PCR in stage 5. Midbrain-specific genes Nurr1, Ptx3, En-1 and dopamine transporter (DAT) are expressed at low levels in the absence of Nurr1 over-expression and Shh/FGF8 treatment at stage 4. (*e*) Nurr1-ES cells integrate into the striatum of hemiparkinsonian rats. Diagram of single sections through a graft ('G') in the striatum (LV, lateral ventricle; AC, anterior commissure). Single confocal images after immunohistochemistry for TH (red) are shown (i–vii) from regions marked by red dots in the diagram. The distribution of cells and processes through the thickness of the section (35 μm) is shown by the Z series displayed in green on the right. Note the many TH+ processes extend away from the graft into the parenchyma of the host striatum (iv–vii). Scale bar, 50 μm. (*f*) Behavioural effects of grafted Nurr1-ES cells. Analysis of amphetamine-stimulated rotations in animals grafted with neurons derived from wt- (red triangles, $n = 10$) or Nurr1-ES cells (green squares, $n = 15$) and sham controls (blue circles, $n = 18$). Reproduced from Kim *et al.* (2002).

There is increasing evidence for the replacement of functional neurons from endogenous cells in the adult CNS. In the dentate gyrus and olfactory bulb, neurogenesis normally occurs at a significant rate (Fuchs & Gould 2000; Cameron & McKay 2001; Van Praag *et al.* 2002). Endogenous cells may also replace neurons in the adult

cortex, striatum and hippocampus after injury (Magavi *et al.* 2000; Benraiss *et al.* 2001; Arvidsson *et al.* 2002; Nakatomi *et al.* 2002). Recent data suggest that endogenous regenerative processes may also be present in the adult substantia nigra (Zhao *et al.* 2003). Our growing understanding of neurogenesis suggests that in the future it may be possible to stimulate neuron production in many sites in the adult brain.

Interest in stem cells has been greatly stimulated by the isolation of human ES cells and their potential use in cell-based therapies (Thomson *et al.* 1998). Mouse ES cells can be induced to generate many cell types including CNS stem cells, haematopoietic stem cells, cardiomyocytes, pancreatic islets and the cells of the vascular system (Yamashita *et al.* 2000; Lumelsky *et al.* 2001; Lacaud *et al.* 2002; Rideout *et al.* 2002). Human ES cells also generate neural fates (Reubinoff *et al.* 2000, 2001; Zhang *et al.* 2001).

The rapid progress in understanding stem cell mechanisms argues that a powerful technology to manipulate human ES cells will open many clinically significant paths. Our own work has encouraged the idea that stem cell technologies might contribute to cell therapies for Parkinson's disease, multiple sclerosis and diabetes. Our understanding of the basic biology of rodent CNS stem cells suggests that human ES cells may also generate midbrain dopamine neurons. Access to large numbers of human dopamine neurons that can be manipulated will promote work by many groups working on different aspects of Parkinson's disease. Their function can be assessed in striatal and nigral grafts. Manipulation of dopaminergic behaviour by grafted human dopamine neurons will provide a sound technical basis for a technology that requires modification before it can be successfully applied in the clinic (Freed *et al.* 2001). The clinical significance of stem cell biology is not restricted to cell replacement and we propose that the ability of stem cell-derived dopamine neurons to form synapses will define new mechanisms controlling dopamine release and neuronal survival. Knowledge of the synaptic and neurotrophic mechanisms of human midbrain dopamine neurons has clear relevance to Parkinson's patients. This example illustrates the general importance of stem cell biology for medicine.

REFERENCES

Altmann, C. R. & Brivanlou, A. H. 2001 Neural patterning in the vertebrate embryo. *Int. Rev. Cytol.* **203**, 447–482.

Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. & Lindvall, O. 2002 Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Med.* **8**, 963–967.

Benraiss, A., Chmielnicki, E., Lerner, K., Roh, D. & Goldman, S. A. 2001 Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *J. Neurosci.* **21**, 6718–6731.

Blondel, O., Collin, C., McCarran, W. J., Zhu, S., Zamostiano, R., Gozes, I., Brenneman, D. E. & McKay, R. D. 2000 A glia-derived signal regulating neuronal differentiation. *J. Neurosci.* **20**, 8012–8020.

Brustle, O., Maskos, U. & McKay, R. D. 1995 Host-guided migration allows targeted introduction of neurons into the embryonic brain. *Neuron* **15**, 1275–1285.

Brustle, O., Jones, K. N., Learish, R. D., Karram, K., Choudhary, K., Wiestler, O. D., Duncan, I. D. & McKay, R. D. 1999 Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science* **285**, 754–756.

Bullock, A. N., Henckel, J. & Fersht, A. R. 2000 Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: definition of mutant states for rescue in cancer therapy. *Oncogene* **19**, 1245–1256.

Cameron, H. A. & McKay, R. D. 2001 Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J. Comp. Neurol.* **435**, 406–417.

Cattaneo, E. & McKay, R. 1990 Proliferation and differentiation of neuronal stem cells. *Nature* **347**, 762–765.

Collin, C., Vicario-Abejon, C., Rubio, M. E., Wenthold, R. J., McKay, R. D. & Segal, M. 2001 Neurotrophins act at presynaptic terminals to activate synapses among cultured hippocampal neurons. *Eur. J. Neurosci.* **13**, 1273–1282.

Davis, A. A. & Temple, S. 1994 A self-renewing multipotential stem cell in embryonic rat cerebral cortex. *Nature* **372**, 263–266.

Frederiksen, K. & McKay, R. D. 1988 Proliferation and differentiation of rat neuroepithelial precursor cells *in vivo*. *J. Neurosci.* **8**, 1144–1151.

Frederiksen, K., Jat, P. S., Valtz, N., Levy, D. & McKay, R. 1988 Immortalization of precursor cells from the mammalian CNS. *Neuron* **1**, 439–448.

Freed, C. R. (and 11 others) 2001 Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *New Engl. J. Med.* **344**, 710–719.

Fuchs, E. & Gould, E. 2000 *In vivo* neurogenesis in the adult brain: regulation and functional implications. *Eur. J. Neurosci.* **12**, 2211–2214.

Hermanson, O., Jepsen, K. & Rosenfeld, M. G. 2002 N-CoR controls differentiation of neural stem cells into astrocytes. *Nature* **419**, 934–939.

Hockfield, S. & McKay, R. D. 1985 Identification of major cell classes in the developing mammalian nervous system. *J. Neurosci.* **5**, 3310–3328.

Isaka, F., Ishibashi, M., Taki, W., Hashimoto, N., Nakanishi, S. & Kageyama, R. 1999 Ectopic expression of the bHLH gene Math1 disturbs neural development. *Eur. J. Neurosci.* **11**, 2582–2588.

Kim, J. H. (and 10 others) 2002 Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* **418**, 50–56.

Lacaud, G., Gore, L., Kennedy, M., Kouskoff, V., Kingsley, P., Hogan, C., Carlsson, L., Speck, N., Palis, J. & Keller, G. 2002 Runx1 is essential for hematopoietic commitment at the hemangioblast stage of development *in vitro*. *Blood* **100**, 458–466.

Le Douarin, N. 1980 Migration and differentiation of neural crest cells. *Curr. Topics Dev. Biol.* **16**, 31–85.

Lee, S. H., Lumelsky, N., Studer, L., Auerbach, J. M. & McKay, R. D. 2000 Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nature Biotechnol.* **18**, 675–679.

Lendahl, U., Zimmerman, L. B. & McKay, R. D. 1990 CNS stem cells express a new class of intermediate filament protein. *Cell* **60**, 585–595.

Lumelsky, N., Blondel, O., Laeng, P., Velasco, I., Ravin, R. & McKay, R. 2001 Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* **292**, 1389–1394.

Magavi, S. S., Leavitt, B. R. & Macklis, J. D. 2000 Induction of neurogenesis in the neocortex of adult mice. *Nature* **405**, 951–955.

Mauch, D. H., Nagler, K., Schumacher, S., Goritz, C., Muller, E. C., Otto, A. & Pfrieger, F. W. 2001 CNS

synaptogenesis promoted by glia-derived cholesterol. *Science* **294**, 1354–1357.

Mitsuhashi, T., Aoki, Y., Eksioglu, Y. Z., Takahashi, T., Bhide, P. G., Reeves, S. A. & Caviness Jr, V. S. 2001 Overexpression of p27Kip1 lengthens the G1 phase in a mouse model that targets inducible gene expression to central nervous system progenitor cells. *Proc. Natl Acad. Sci. USA* **98**, 6435–6440.

Mozhayeva, M. G., Sara, Y., Liu, X. & Kavalali, E. T. 2002 Development of vesicle pools during maturation of hippocampal synapses. *J. Neurosci.* **22**, 654–665.

Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T. & Nakafuku, M. 2002 Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* **110**, 429–441.

Nieuwkoop, P. 1952 Activation and organization of the amphibian central nervous system. *J. Exp. Zool.* **12**, 1–130.

Panchision, D. M., Pickel, J. M., Studer, L., Lee, S. H., Turner, P. A., Hazel, T. G. & McKay, R. D. 2001 Sequential actions of BMP receptors control neural precursor cell production and fate. *Genes Dev.* **15**, 2094–2110.

Petersen, P. H., Zou, K., Hwang, J. K., Jan, Y. N. & Zhong, W. 2002 Progenitor cell maintenance requires numb and numblike during mouse neurogenesis. *Nature* **419**, 929–934.

Ready, D., Hanson, T. E. & Benzer, S. 1976 Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53**, 217–240.

Renfranz, P. J., Cunningham, M. G. & McKay, R. D. 1991 Region-specific differentiation of the hippocampal stem cell line HiB5 upon implantation into the developing mammalian brain. *Cell* **66**, 713–729.

Reubinoff, B. E., Pera, M. F., Fong, C. Y., Trounson, A. & Bongso, A. 2000 Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nature Biotechnol.* **18**, 399–404.

Reubinoff, B. E., Itsykson, P., Turetsky, T., Pera, M. F., Reinhartz, E., Itzik, A. & Ben-Hur, T. 2001 Neural progenitors from human embryonic stem cells. *Nature Biotechnol.* **19**, 1134–1140.

Reynolds, B. A. & Weiss, S. 1992 Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707–1710.

Rideout, W. M., Hochedlinger, K., Kyba, M., Daley, G. Q. & Jaenisch, R. 2002 Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell* **109**, 17–27.

Song, H. J., Stevens, C. F. & Gage, F. H. 2002 Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nature Neurosci.* **5**, 438–445.

Studer, L., Tabar, V. & McKay, R. D. 1998 Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. *Nature Neurosci.* **1**, 290–295.

Sun, Y., Nadal-Vicens, M., Misono, S., Lin, M. Z., Zubiaga, A., Hua, X., Fan, G. & Greenberg, M. E. 2001 Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. *Cell* **104**, 365–376.

Temple, S. 1989 Division and differentiation of isolated CNS blast cells in microculture. *Nature* **340**, 471–473.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. & Jones, J. M. 1998 Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147.

Tsai, R. Y. & McKay, R. 2002 Nucleolar control of cell proliferation in stem cells and cancer cells. *Genes Dev.* **16**, 2991–3003.

Turnley, A. M., Faux, C. H., Rietze, R. L., Coonan, J. R. & Bartlett, P. F. 2002 Suppressor of cytokine signaling 2 regulates neuronal differentiation by inhibiting growth hormone signaling. *Nature Neurosci.* **5**, 1155–1162.

Van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D. & Gage, F. H. 2002 Functional neurogenesis in the adult hippocampus. *Nature* **415**, 1030–1034.

Vicario-Abejon, C., Cunningham, M. G. & McKay, R. D. 1995 Cerebellar precursors transplanted to the neonatal dentate gyrus express features characteristic of hippocampal neurons. *J. Neurosci.* **15**, 6351–6363.

Vicario-Abejon, C., Collin, C., Tsoulfas, P. & McKay, R. D. 2000 Hippocampal stem cells differentiate into excitatory and inhibitory neurons. *Eur. J. Neurosci.* **12**, 677–688.

Wichterle, H., Lieberam, I., Porter, J. A. & Jessell, T. M. 2002 Directed differentiation of embryonic stem cells into motor neurons. *Cell* **110**, 385–397.

Yamashita, J., Itoh, H., Hirashima, M., Ogawa, M., Nishikawa, S., Yurugi, T., Naito, M., Nakao, K. & Nishikawa, S. 2000 Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature* **408**, 92–96.

Zhang, S. C., Wernig, M., Duncan, I. D., Brustle, O. & Thomson, J. A. 2001 *In vitro* differentiation of transplantable neural precursors from human embryonic stem cells. *Nature Biotechnol.* **19**, 1129–1133.

Zhao, M., Momma, S., Delfani, K., Carlen, M., Cassidy, R. M., Johansson, C. B., Brismar, H., Shupliakov, O., Frisen, J. & Janson, A. M. 2003 Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc. Natl Acad. Sci. USA* **100**, 7925–7930.

GLOSSARY

BMP: bone morphogenetic protein
CNS: central nervous system
ES: embryonic stem
NS: nucleostemin
TH: tyrosine hydroxylase
wt: wild-type